

THE NUTRITIVE RÔLE OF CERTAIN AMINO ACIDS

BY

ALECK BORMAN

B. S., University of Toledo, 1941

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE SCHOOL OF THE
UNIVERSITY OF ILLINOIS, 1945

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THE NUTRITIVE ROLE OF CERTAIN AMINO ACIDS

PART I

THE POSSIBLE REPLACEMENT OF *l*(+)-ARGININE BY ARGININIC ACID, *d*(—)-ARGININE, AND *l*(+)-ORNITHINE

INTRODUCTION

The synthesis of arginine in the growing rat has been well established by Scull and Rose (1). However, it has been shown by Gunther and Rose (2) that animals deprived of this amino acid gain only seventy to eighty per cent as much as controls receiving arginine. Therefore arginine is indispensable for the growing rat, since it cannot be synthesized, out of materials ordinarily in the diet, at a speed commensurate with the demands for normal growth (Rose, 3). It has already been established in this laboratory that citrulline can replace arginine while ornithine cannot (4,5,6). The results with ornithine are not in agreement with the theory of Krebs and Henseleit for the formation of urea (7). According to the work of these investigators, both ornithine and citrulline should accelerate the growth of rats on an arginine-free diet.

This question has been reinvestigated in the present study. For this purpose a new diet was used. This provided a much greater difference in growth rate on rations devoid of arginine and those containing this amino acid than have ever been obtained in any of the previous experiments. In addition, the possible replacement of arginine by argininic acid and by the unnatural optical isomer has been studied, using both the improved diet and the diet previously used in this laboratory.

EXPERIMENTAL

Young albino rats weighing from 42 to 63 gm. received diets which were devoid of protein but contained a mixture of highly purified amino acids. Both the older diets with a high fat content and the improved rations containing only two per cent fat were

used. The *l*(+)-arginine of the ration was replaced by argininic acid, *l*(+)-ornithine, *d*(—)-arginine, or *dl*-arginine. In addition, a paired-feeding experiment was conducted, comparing a ration containing arginine with one minus this amino acid. Each experiment was carried out for twenty-eight days.

The results clearly indicate that the rats receiving argininic acid, ornithine, the unnatural isomer of arginine, or racemic arginine did not grow nearly so rapidly as the control animals receiving the natural form of arginine. When the food intake was restricted, the animals on a ration containing arginine grew no better than those on a diet devoid of this amino acid.

SUMMARY

Arginine cannot be replaced in the diet of growing rats by argininic acid, ornithine or the unnatural isomer of arginine.

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PART II

THE NUTRITIVE VALUE OF CERTAIN HYDROXY DERIVATIVES OF THREONINE, VALINE, AND PHENYLALANINE

INTRODUCTION

During the course of a growth experiment designed to test the replaceability of threonine by *d*-threo- α -amino- β , γ -dihydroxy-*n*-butyric acid (γ -hydroxythreonine), it was found that the latter compound is fatally toxic to the animals. Because of this unusual result it was thought to be of interest to examine other essential amino acids whose structure was altered by placing an hydroxyl group on the carbon atom adjacent to one holding another functional group in the molecule. β -hydroxyleucine

was examined by others in this laboratory (1) and found to be highly toxic, killing all animals which received it. Equivalent amounts of added leucine had no effect in alleviating the symptoms. In the present study β -hydroxyvaline and β -hydroxyphenylalanine (phenylserine) were tested. The effect of adding large amounts of valine and phenylalanine to the diets containing the corresponding β -hydroxy derivatives was also investigated.

EXPERIMENTAL

Albino rats were used as in Part I. The diets were devoid of protein but contained a mixture of highly purified amino acids. When γ -hydroxythreonine replaced threonine in the ration, the animals lost weight and died within 10 to 20 days. If β -hydroxyvaline was substituted for valine in the diet, again the rats died within 14 to 19 days. The addition of 4 or 16 per cent of *dl*-valine to this toxic ration produced diets which displayed no toxicity. When the phenylalanine of the ration was displaced by an equivalent amount of phenylserine, the animals showed signs of intoxication, half of them dying within the twenty-eight day period. The addition of 2.4 or 6 per cent of *dl*-phenylalanine to the toxic ration completely eradicated all harmful effects of the hydroxy compound.

Toxic symptoms were found in all animals on diets containing any of these hydroxy compounds unsupplemented by excessive amounts of the corresponding amino acid. These included semi-paralysis of the hind limbs and hemorrhages around the eyes, nose, and feet, becoming more pronounced until the animals died. Negative controls, ingesting neither the amino acid nor its corresponding hydroxy derivative, exhibited no toxic symptoms and lost less weight than the animals on the deleterious rations.

SUMMARY

The substituting of equivalent amounts of γ -hydroxythreonine for threonine, β -hydroxyvaline for valine, or phenylserine for phenylalanine, respectively, in the diets of growing rats resulted in the production of fatally toxic rations. The addition of excessive amounts of valine and phenylalanine, respectively, to the latter two rations completely removed all signs of intoxica-

tion. The mechanism may involve a competition for the enzymes associated with amino acid anabolism.

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PART III

THE LYSINE REQUIREMENT FOR THE MAINTENANCE OF NITROGEN EQUILIBRIUM IN MAN

INTRODUCTION

The previous use of nitrogen balance studies in this laboratory has shown that eight amino acids are essential for the maintenance of nitrogen equilibrium in man (1, 2, 3). These are lysine, tryptophane, phenylalanine, leucine, isoleucine, threonine, methionine, and valine. The quantitative requirement of tryptophane has already been established (4). The recommended daily intake is 200-250 mgm. It was also found that the unnatural optical isomer of tryptophane cannot be utilized by man.

In the present study the quantitative human requirement for lysine has been determined and the unnatural isomer tested. In addition, a report by Albanese, Holt, et al. (5) concerning the increased urinary excretion of organic acids during lysine deficiencies has prompted a further investigation of this phenomenon.

EXPERIMENTAL

Three adult male subjects were used in these experiments. The diets contained 10 gm. of nitrogen, of which over ninety-five per cent was supplied in the form of purified amino acids and urea. The other constituents of the rations were corn starch, sucrose, butter, cellu flour, corn oil, minerals, lemon juice, haliver oil concentrate, and a mixture of vitamins. Distilled water was taken *ad libitum*. The caloric intake remained constant throughout each experiment. It had been previously demonstrated that 55 calories per kilogram of body weight were necessary for the maintenance of positive nitrogen balance on a complete synthetic diet of this nature. It was found during the present investigations that the caloric intake should exceed 2000 calories per square

meter of body surface and that the ratio of calories of carbohydrate to calories of fat in the ration should be greater than 2.

Twenty-four hour urine samples were collected and preserved with toluene. Daily analyses were run for total nitrogen, creatinine, and organic acids. The feces were collected separately for each dietary period by means of charcoal markers. They were dried, mixed, and analyzed for total nitrogen. A control diet was administered to each subject until several consecutive days of positive nitrogen balance were obtained. The lysine content of the ration was then lowered and the nitrogen balance noted for several days. This procedure was continued until a period of negative balance was obtained. At this point the lysine level was increased until positive nitrogen balance was again achieved. The lowering and raising of the lysine content of the diet was continued until a response to a change of 0.1 gm. per day of this amino acid was observed. The minimum daily requirement is defined as *the lowest level of an amino acid necessary to maintain consistent positive nitrogen balance.*

Using this criterion, the minimum daily level of lysine was established as 400 mgm. To allow for individual variations, an intake of 600 mgm. of lysine per day is recommended. The unnatural optical isomer of this amino acid cannot be utilized by man. No variation in daily organic acid excretion was noted that could be attributed to the lysine content of the ration.

SUMMARY

The minimum daily amount of lysine for the maintenance of nitrogen equilibrium in man has been determined. A daily intake of 600 mgm. is recommended. The unnatural isomer of lysine cannot be utilized. The urinary excretion of organic acids is not affected by the lysine content of the ration.

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VITA

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